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# Prospective Evaluation of Ursodeoxycholic Acid Withdrawal in Patients With Primary Sclerosing Cholangitis

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**Ursodeoxycholic acid (UDCA) is no longer recommended for management of adult patients with primary sclerosing cholangitis (PSC). We undertook a prospective evaluation of UDCA withdrawal in a group of consecutive patients with PSC. Twenty six patients, all treated with UDCA (dose range: 10-15 mg/kg/day) were included. Paired blood samples for liver biochemistry, bile acids, and fibroblast growth factor 19 (FGF19) were collected before UDCA withdrawal and 3 months later. Liquid chromatography/tandem mass spectrometry was used for quantification of 29 plasma bile acid metabolites. Pruritus and health-related quality of life (HRQoL) were assessed with a 10-point numeric rating scale, the Medical Outcomes Study Short Form-36 (SF-36), and PBC-40 questionnaires. UDCA withdrawal resulted in a significant deterioration in liver biochemistry (increase of alkaline phosphatase of 75.6%; $P < 0.0001$; gamma-glutamyl transpeptidase of 117.9%, $P < 0.0001$; bilirubin of 50.0%, $P < 0.001$; alanine aminotransferase of 63.9%, $P < 0.005$; and aspartate aminotransferase of 45.0%, $P < 0.005$) and increase of Mayo Risk Score for PSC (change from baseline of +0.5 point; $P < 0.003$). Bile acid analysis revealed a significant decrease in lithocholic acid and its derivatives after UDCA withdrawal, but no effect on concentrations of primary bile acids aside from an increased accumulation of their taurine conjugates. After UDCA removal cholestatic parameters, taurine species of cholic acid and chenodeoxycholic acid correlated with serum FGF19 levels. No significant effect on HRQoL after UDCA withdrawal was observed; however, 42% of patients reported a deterioration in their pruritus. *Conclusion:* At 3 months, discontinuation of UDCA in patients with PSC causes significant deterioration in liver biochemistry and influences concentrations of bile acid metabolites. A proportion of patients report increased pruritus, but other short-term markers of quality of life are unaffected. (HEPATOLOGY 2014;60:931-940)**

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Primary sclerosing cholangitis (PSC) is a slowly progressive autoimmune biliary disease frequently observed in association with inflammatory bowel disease (IBD).[1] Progression to end-stage liver disease remains common, with no effective pharmacologic intervention.[2] Most trial data have evaluated the effect of ursodeoxycholic acid (UDCA), a dihydoxy bile acid, naturally comprising 2% of the human bile acid pool. UDCA possesses several

*Abbreviations: AASLD, American Association for the Study of Liver Diseases; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; FGF19, fibroblast growth factor 19; GGT, gamma-glutamyl transpeptidase; HCA, hyocholic acid; HDCA, hyodeoxycholic acid; HRQoL, health-related quality of life; IBD, inflammatory bowel disease; IgG4, immunoglobulin G4; INR, international normalized ratio; LCA, lithocholic acid; LC-MS/MS, liquid chromatography/tandem mass spectrometry; MRS, Mayo Risk Score; NRS, numeric rating scale; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; SD, standard deviation; SAEs, serious adverse events; SF-36, the Medical Outcomes Study Short Form-36; UDCA, ursodeoxycholic acid; ULN, upper limit of normal.*

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hepatoprotective properties, which have been argued as being beneficial in chronic cholestasis.[3] Though largely considered effective in primary biliary cirrhosis (PBC),[4,5] efficacy in PSC is controversial and lately its use actively discouraged. A recent study by Lindor et al. showed that long-term, high-dose therapy with UDCA did not improve survival and was related to higher rates of serious adverse events (SAEs).[6] It was speculated that these results may reflect the harmful effect of toxic bile acids produced from high levels of UDCA in the colon.[7] The American Association for the Study of Liver Diseases (AASLD) currently recommends against UDCA use in patients with PSC,[8] whereas the European Association for the Study of the Liver Guidelines suggested that UDCA be only offered to patients with PSC who also have advanced colitis.[9]

In this study, we prospectively analyzed the effect of UDCA withdrawal on various clinical, laboratory, and quality-of-life parameters in a cohort of well-characterized patients with PSC. Given the previous reported influence of UDCA on bile acid homeostasis, we also evaluated plasma bile acid metabolites and fibroblast growth factor 19 (FGF19). FGF19, an endocrine factor produced by the small intestine in response to uptake of bile salts, has a role in negative feedback regulation of hepatic bile acid synthesis, as well as protecting the liver against bile salt toxicity.[10] Transcription of human FGF19 has been shown to be stimulated by several bile acids[11]; however, the specific effect of UDCA has, as yet, not been studied.

## Patients and Methods

***Patients.*** Twenty-nine patients with PSC established by EASL criteria (i.e., elevated serum markers of cholestasis and typical bile duct changes in magnetic resonance or endoscopic cholangiography[9]) were included. Immunoglobulin G4 (IgG4)-related cholangitis was excluded. None of the included subjects suffered from other conditions that could significantly influence health-related quality of life (HRQoL), such as decompensated diabetes mellitus, renal insufficiency requiring dialyses, malignancy, heart failure of at least New York Heart Association class II, rheumatoid arthritis, or asthma. All patients were treated with UDCA (10-15 mg/kg/day) for at least 12 months before withdrawal. Clinical data, HRQoL questionnaires, and paired blood samples for liver biochemistry, FGF19, and bile acids were collected 1 day before UDCA withdrawal and 3 months after. Three patients were excluded from further analysis because of severe complications of liver disease and inability to perform follow-up examination (1 male, variceal bleeding; 1 female, decompensation of liver cirrhosis). The third patient experienced a gradual increase of pruritus after UDCA withdrawal, which became unbearable 3 weeks after UDCA was stopped. He resumed UDCA on his own and his itching subsequently decreased.

Thus, a group of 26 patients was analyzed: 16 (62%) males and 10 (38%) females, with a mean age of $33.9 \pm 10.5$ years. Five (19%) patients were diagnosed with cirrhosis by liver biopsy or imaging studies. Eighteen subjects (69%) suffered from IBD, including 13 (50%) with ulcerative colitis and 5 (19%) with nonclassified colitis. Demographic and clinical data on analyzed patients are summarized in Table 1.

***Bile Acid Determination.*** Blood samples for bile acid determination were collected in ethylenediaminetetraacetic acid tubes. Immediately after collection, plasma was purified through centrifugation at 4°C. Subsequently, one volume of formic acid (0.5 M) was added to plasma samples. Samples were frozen and kept at −80°C until processed. Bile acids were purchased from Steraloids (Newport, RI), whereas glucuronidated acids were produced as previously reported.[12] Deuterated isotopes used as analytical standards were from C/D/N Isotopes, Inc. (Pointe-Claire, Québec, Canada). For bile acid/glucuronide metabolites, deuterated standards were synthesized as previously described.[12] All chemicals and solvents were of the highest grade. Methanol, ethyl acetate, hexane, isooctane, 1-chlorobutane, and isoamyl alcohol were obtained from VWR (Montréal, Québec, Canada). Pyridine was purchased from Regis Technologies (Morton Grove, IL). Ammonium hydroxide, citric acid, and acetic acid were obtained from Fisher Scientific (Ottawa, Ontario, Canada). Other reagents were purchased from Sigma-Aldrich Co (Oakville, Ontario, Canada). Solid-phase extraction columns were from Phenomenex (Torrance, CA), Varan Inc. (Palo Alto,

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**Table 1. Clinical and Laboratory Characteristics of Study Patients at Entry**

| Feature | Results (n = 26) |
|---|---|
| Age, years | 33.9 ± 10.5 |
| Gender, M (%)/F (%) | 16 (62)/10 (38) |
| IBD, none/UC/nonclassified (%) | 8 (31)/13 (50)/5 (19) |
| Cirrhosis, yes/no (%) | 5 (19)/21 (81) |
| Hemoglobin, mg/dL | 13.2 ± 2.2 |
| ALT, IU/L (normal: <30) | 80.9 ± 77.2 |
| AST, IU/L (normal: <30) | 67.3 ± 51.6 |
| ALP, IU/L (normal: <120) | 250.0 ± 210.0 |
| GGT, IU/L (normal: <42) | 301.2 ± 377.1 |
| Bilirubin, mg/dL (normal: <1.0) | 1.6 ± 3.6 |
| Albumin, g/dL (normal: 3.8-4.4) | 4.4 ± 0.4 |
| INR (normal: 0.8-1.2) | 1.1 ± 0.1 |
| Ca-19.9, IU/mL (normal: <18.4) | 13.9 ± 20.2 |
| IgG4, g/L (normal: 0.03-2.01) | 1.21 ± 0.81 |

Data shown as mean ± SD.

Abbreviations: M, male; F, female; UC, ulcerative colitis; IBD, inflammatory bowel disease.

CA), or Waters (Milford, MA). Unconjugated, taurine-, glycine- sulfate-, and glucuronide-conjugated bile acid levels were measured by validated liquid chromatography/tandem mass spectrometry (LC-MS/MS) methods.[12-15] Lower limit of quantification varied from 1.0 (LCA-3G) to 8.0 nM (TCDCA), as previously reported.[12-15]

***Determination of FGF19.*** Serum FGF19 levels were determined using a sandwich enzyme-linked immunosorbent assay specific for FGF19 (R&D Systems, Minneapolis, MN).

***Assessment of Quality of Life and Pruritus.*** A 10-point numeric rating scale (NRS) was applied for assessment of pruritus intensity (0 points, no pruritus; 10 points, the most intense pruritus they can imagine).[16] HRQoL was assessed with both generic (the Medical Outcomes Study Short Form-36; SF-36) and disease-specific questionnaires (PBC-40). The PBC-40 was constructed for evaluation of HRQoL in patients with PBC[17] and, more recently, has been used in patients with PSC.[18] The PBC-40 contains 40 questions in the following domains: fatigue, cognitive, social-emotional, itch, and other symptoms, with higher scores indicating poorer HRQoL. The SF-36 is a widely used and validated HRQoL questionnaire, which includes 36 items divided into eight scales. Scores can be obtained for each scale or can be aggregated into two summary scores: a Mental Component Summary and a Physical Component Summary score. Scale scores range between 0 and 100, with the higher score indicating better HRQoL.[19] A license No. QM011392-QualityMetric CT133208/OP018661 was obtained for the use of the SF-36 questionnaire in this study. One patient did not fill the SF-36 questionnaire at inclusion; thus, the final HRQoL analysis comprised of 25 paired SF-36 and 26 paired PBC-40 questionnares.

***Ethics.*** Written informed consent was obtained from each patient included in the study. The study protocol was approved by the ethics committee of Pomeranian Medical University (Szczecin, Poland) and conformed to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008).

***Statistical Analysis.*** Data were evaluated as mean ± standard deviation (SD) for continuous variables. Serum bile acid concentrations did not satisfy the normal distribution, according to Shapiro-Wilk's test, thus Wilcoxon's matched-pairs signed-rank test was used for statistical analyses of response to treatment. Comparisons of response to treatment between men and women were made using Wilcoxon's/Mann-Whitney's rank-sum test (JMP V7.0.1; SAS Institute Inc., Cary, NC). Effect of treatment withdrawal was determined as the difference between values after withdrawal and values before. Correlations were assessed both by parametric and nonparamatric (Spearman's rank-correlation coefficient) tests using the JMP Statistical Discovery program (V7.0.1; SAS Institute). A multivariate analysis was performed using step-wise regression models. A $P$ value $<0.05$ was considered statistically significant.

## Results

***Liver Biochemistry Tests and Mayo Risk Score.*** Laboratory findings of analyzed patients before UDCA withdrawal are shown in Table 1. At entry, 18 patients (69.2%) had elevated alkaline phosphatase (ALP). UDCA withdrawal resulted in a highly significant elevation in cholestatic parameters (increase of ALP of 75.6% to 438.6 ± 315.9 IU/L, $P < 0.0001$; gamma-glutamyl transpeptidase [GGT] of 117.9% to 656.3 ± 553.5 IU/L, $P < 0.0001$; bilirubin of 50.0% to 2.4 ± 4.5 mg/dL, $P < 0.001$). In effect, whereas at baseline, 12 (46.1%) patients on UDCA had ALP <1.5× upper limit of normal (ULN), at 3 months after discontinuation of UDCA, only 8 (30.8%) had ALP <1.5× ULN. In 5 patients, we did not observe any deterioration in ALP, and, of note, 4 of them had normal ALP values at entry. With respect to aminotransferases, there was a significant increase in both alanine aminotransferase (ALT) of 63.9% to 132.6 ± 100.2 IU/L ($P < 0.005$) and aspartate aminotransferase (AST) of 45.0% to 97.6 ± 66.5 IU/L ($P < 0.005$; Fig. 1A-E). No changes in serum albumin levels or international normalized ratio (INR) were noted (data not shown). The calculated Mayo Risk

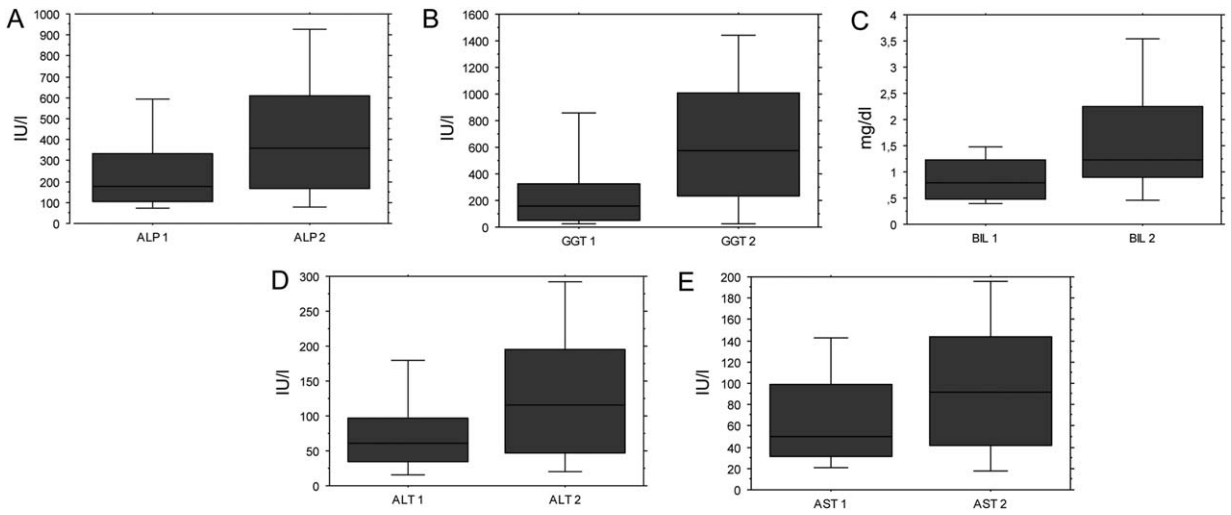


Fig. 1. Changes in liver biochemistry tests after UDCA withdrawal with respect to (A) ALP, (B) GGT, (C) total bilirubin, (D) ALT, and (E) AST. 1, Results of individual tests before UDCA withdrawal. 2, Results of individual tests 3 months after UDCA withdrawal.

Score (MRS) for PSC[20] showed a significant increase at the end of the study, in comparison with the day of enrollment ($-0.2 \pm 1.0$ vs. $-0.7 \pm 0.9$, respectively; $P < 0.003$). There was no association between duration of UDCA treatment before enrollment, age, or gender and changes of liver biochemistry tests (data not shown).

***Bile Acid Pool.*** The main component of serum bile acid pool in patients treated with UDCA was the glycine conjugate of UDCA (40.6%). Together with the unconjugated form of UDCA and its taurine derivative, they represented almost half of the total bile acid pool (49.8%). The majority remaining (47.1%) were primary bile acids, among them glycine conjugates of cholic acid (CA; 13.9%) and chenodeoxycholic acid (CDCA; 16.0%). The main secondary bile acid was glycine conjugate of deoxycholic acid (DCA; 1.7% of total pool). The concentration of lithocholic acid (LCA) and its conjugates was $168.3 \pm 36.9$ nM and represented 0.3% of the total pool (Fig. 2A). UDCA discontinuation resulted, as expected, in changes both in total concentration and in profile of serum bile acids (Fig. 2B). This was mainly caused by a marked decrease of UDCA ($P < 0.0001$) and its taurine ($P < 0.0001$) and glycine ($P < 0.0001$) conjugates. This resulted in UDCA and its derivatives reducing to 1.7% of the total pool. A significant decrease of LCA ($P < 0.0001$), sulpholithocholate ($P = 0.0001$), glycine ($P = 0.0003$), taurine ($P = 0.0366$), and glucuronidated species of LCA (LCA-3G and LCA-24G; $P = 0.0005$ and $P < 0.0001$, respectively) was also observed. However, the concentration of LCA and its conjugates was $50.7 \pm 9.6$ nM and still had little proportional (0.2%) contribution to the total bile acid pool. No effect on CA, CDCA, and DCA concentrations and their glycine conjugates were observed after UDCA withdrawal; however, an accumulation of their taurine conjugates ($P < 0.02$ for all) was observed. Primary and secondary bile acids represented 97.7% total bile acids after UDCA removal. A significant reduction of 24-*O*-glucuronides of CDCA ($P < 0.003$) and 6-*O*-glucuronides of CDCA ($P < 0.02$), but increase in 3-*O*-glucuronides of CDCA ($P < 0.03$), were also noted. We did not observe any significant difference in hyocholic acid (HCA) and hyodeoxycholic acid (HDCA) concentrations. Molar concentrations of bile acids before and after UDCA removal are shown in Table 2 and percentage proportions of the analytes are shown in Fig. 3. IBD and liver cirrhosis had no significant effect on bile acid concentrations both before and after withdrawal (data not shown).

***FGF19.*** Circulating FGF19 levels were not statistically different before and after UDCA withdrawal (99.6 vs. 164.4 pg/mL; $P = 0.3$). No correlation between FGF19 levels and liver biochemistry tests and total/all single bile acids concentrations were observed in UDCA-treated patients (data not shown). After UDCA removal, FGF19 correlated with cholestatic parameters (i.e., ALP and total bilirubin), total bile acid concentration, as well as glycine and taurine conjugates of CA and CDCA (Table 3). In the multivariate analysis, taurine species of CA and CDCA were independent variables related to serum FGF19 levels ($P < 0.0001$).

***Quality of Life.*** UDCA withdrawal resulted in diverse changes in HRQoL questionnaires. A trend toward deterioration of HRQoL in some domains of

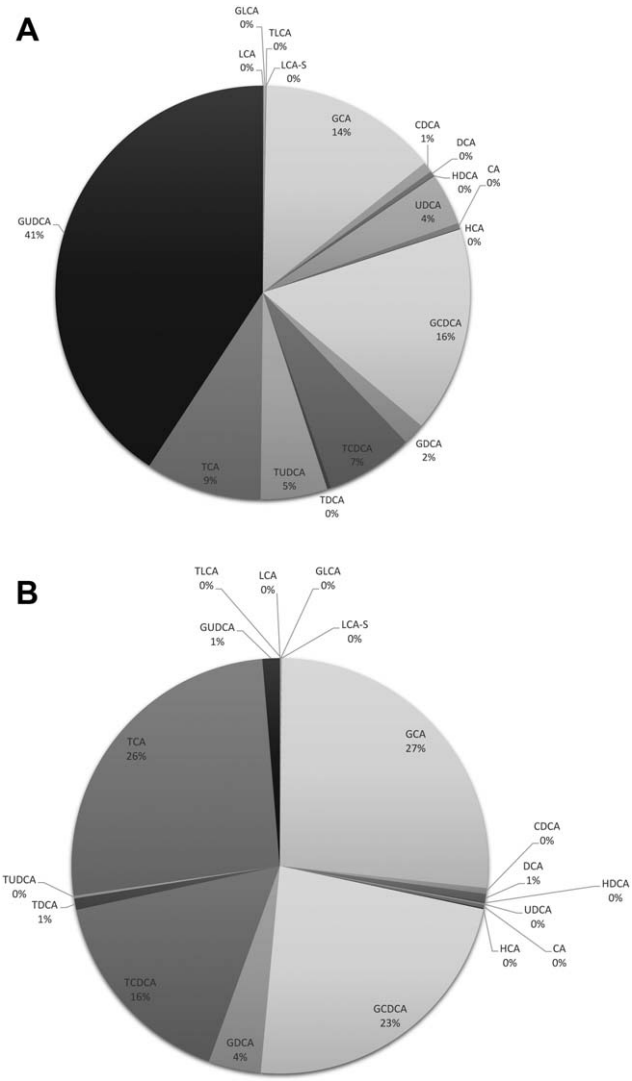


Fig. 2. Mean percentages of bile acids under UDCA treatment (A) and after drug removal (B). Abbreviations: UDCA, GUDCA, TUDCA, ursodeoxycholic acid and its glycine and taurine conjugates, respectively; LCA, GLCA, TLCA, lithocholic acid and its glycine and taurine conjugates, respectively; LCA-S, sulpholithocholate; CA, GCA, TCA, cholic acid and its glycine and taurine conjugates, respectively; CDCA, GDCA, TGCDA, chenodeoxycholic acid and its glycine and taurine conjugates, respectively; DCA, GDCA, TDCA, deoxycholic acid and its glycine and taurine conjugates, respectively.

SF-36 (Role-Physical, General Health, Vitality, and Mental Heath) and the PBC-40 questionnaire (Itch, Fatigue, Social, and Emotional) was noted; however, these differences did not reach statistical significance (Table 4). Of interest, included patients reported an improvement of well-being in the Social Functioning domain and Mental Component Summary in the SF-36 (Table 4); however, there was a proportion of patients who reported an impairment in disease-related symptoms 3 months after UDCA withdrawal (Table 5). Mean NRS on UDCA was $0.5 \pm 1.2$ points, in comparison with $0.9 \pm 2.1$ points at the end of the study ($P = 0.2$); however, when pruritus was assessed with the itching domain of the PBC-40 score, 11 patients (42%) reported worsening of their pruritus.

## Discussion

UDCA has been repeatedly evaluated for management of patients with PSC.[21-25] As with PBC, the studies and the evidence have been conflicting at times, but with the publication of the Lindor at al. high-dose UDCA trial in PSC, and its reported deleterious findings,[6] the AASLD guidelines proposed that UDCA no longer had a role in PSC management.[8] At the same time, a number of clinical trial agents are being proposed for patients with PSC, some of which are being trialed in settings where patients need to have stopped UDCA. Therefore, there is a need for evaluation of patient impact of withdrawing from UDCA in established PSC patients under routine review.

In this present study, we, for the first time, evaluated prospectively the effect of UDCA withdrawal in PSC, including changes to liver biochemistry, serum bile acids, and FGF19 profiles, quality of life, and, finally, survival probability measured by the MRS. In analyzed patients, UDCA withdrawal resulted in a highly significant deterioration of liver biochemistry and increased MRS of 0.5 point. In bile acid profiles, we observed a significant decrease of LCA and its derivatives and accumulation of taurine species of primary bile acids. After UDCA removal, cholestatic parameters as well as conjugates of CA and CDCA correlated with serum FGF19 levels. Short-term markers of quality of life were mostly unaffected; however, there was an improvement of well-being in the Social Functioning domain and Mental Component Summary in the SF-36. Nevertheless, there was a proportion of patients who reported an impairment in disease-related symptoms, and 42% of patients reported increased pruritus measured by the itching domain of the PBC-40.

The beneficial effect of UDCA, in terms of liver biochemistry in naïve PSC patients, has been described for some time. In contrast to this previous experience, we, for the first time, evaluated prospectively the effect of UDCA withdrawal in patients with PSC. Our study clearly shows that interruption of long-term treatment with UDCA causes a significant deterioration of liver biochemistry, including both cholestatic parameters and aminotransferases. Three months after UDCA discontinuation, the proportion of patients who had an

**Table 2. Effect of UDCA Withdrawal on Bile Acid Molar Concentrations**

| | On UDCA (nM ± SD) | After Withdrawal (nM ± SD) | *P* Value |
|---|---|---|---|
| UDCA | 2,280.35 ± 3,396.67 | 39.83 ± 64.86 | <0.0001 |
| GUDCA | 22,946.30 ± 44,018.30 | 429.30 ± 1 405.90 | <0.0001 |
| TUDCA | 2,931.31 ± 6,965.42 | 66.40 ± 173.30 | <0.0001 |
| LCA | 33.59 ± 31.16 | 10.95 ± 11.48 | <0.0001 |
| GLCA | 100.09 ± 135.29 | 26.60 ± 26.23 | <0.0005 |
| TLCA | 16.35 ± 33.31 | 6.49 ± 5.94 | <0.04 |
| LCA-S | 6.68 ± 7.86 | 1.46 ± 2.30 | 0.0001 |
| LCA-3G | 6.78 ± 8.34 | 3.41 ± 5.03 | 0.0005 |
| LCA-24G | 4.82 ± 4.05 | 1.79 ± 1.95 | <0.0001 |
| CA | 252.27 ± 556.72 | 86.55 ± 119.62 | 0.2556 |
| GCA | 7,856.27 ± 16,257.60 | 8,508.72 ± 16,430.40 | 0.3253 |
| TCA | 5,094.28 ± 12,360.50 | 8,315.34 ± 20,654.30 | 0.0156 |
| CA-24G | 6.25 ± 11.82 | 12.95 ± 35.04 | 0.6123 |
| CDCA | 457.06 ± 1,057.34 | 147.94 ± 267.44 | 0.3130 |
| GCDCA | 9,058.61 ± 16,039.00 | 7,408.47 ± 11,193.50 | 0.4646 |
| TCDCA | 3,837.53 ± 7,172.22 | 5,151.90 ± 9,262.45 | 0.0121 |
| CDCA-3G | 93.78 ± 88.59 | 134.04 ± 141.22 | 0.0231 |
| CDCA-24G | 2.57 ± 4.69 | 0.94 ± 1.43 | 0.0028 |
| DCA | 212.65 ± 232.92 | 209.07 ± 255.91 | 0.7203 |
| GDCA | 966.51 ± 1 517.41 | 1,299.61 ± 3,533.85 | 0.8859 |
| TDCA | 157.37 ± 269.95 | 285.36 ± 398.61 | 0.0198 |
| DCA-3G | 26.79 ± 45.03 | 26.24 ± 39.16 | 0.9902 |
| DCA-24G | 2.85 ± 2.39 | 2.41 ± 2.74 | 0.1564 |
| HDCA | 38.43 ± 76.76 | 21.25 ± 27.19 | 0.4386 |
| HDCA-6G | 64.94 ± 81.01 | 36.63 ± 46.01 | 0.0198 |
| HDCA-24G | 1.16 ± 0.88 | 0.98 ± 0.51 | 0.2969 |
| HCA | 25.15 ± 38.88 | 19.26 ± 24.52 | 0.7203 |
| HCA-6G | 92.22 ± 114.64 | 89.10 ± 91.55 | 0.7389 |
| HCA-24G | 0.24 ± 0.22 | 0.29 ± 0.18 | 0.3258 |

All data shown as mean ± SD.

Abbreviations: UDCA, GUDCA, TUDCA, ursodeoxycholic acid and its glycine and taurine conjugates, respectively; LCA, GLCA, TLCA, LCA-3G, LCA-24G, lithocholic acid and its glycine, taurine, and glucuronide conjugates, respectively; LCA-S, sulpholithocholate; CA, GCA, TCA, CA-24G, cholic acid and its glycine, taurine, and glucuronide conjugates, respectively; CDCA, GCDCA, TCDCA, CDCA-3G, CDCA-24G, chenodeoxycholic acid and its glycine, taurine, and glucuronide conjugates, respectively; DCA, GDCA, TDCA, DCA-3G, DCA-24G, deoxycholic acid and its glycine, taurine, and glucuronide conjugates, respectively; HDCA, HDCA-6G, HDCA-24G, hyodeoxycholic acid and its glucuronide conjugates; HCA, HCA-6G, HCA-24G, hyocholic acid and its glucuronide conjugates.

ALP <1.5× ULN, a stratifier proposed recently as predicting good outcome in PSC,[26] decreased. Of interest, in the small proportion of patients in whom ALP did not increase after withdrawal, ALP values at entry of the study were normal.

Changes in liver biochemistry after UDCA withdrawal were followed by an increase in MRS by 0.5 point; nevertheless, the calculated mean score did not exceed 0.0 ("low risk" group) at the end of the study. This can be explained by the fact that the majority of included patients had a relatively stable course of disease and initially low risk (stratification by the Mayo model) before enrollment. During the study, we did not observe SAEs and the calculated difference was mostly caused by aminotransferases and bilirubin elevation. Whether this statistically significant increase in MRS truly indicates the possible negative influence of withdrawal on survival is not clear at this point.

One hypothesis for why there may be harmful effects of UDCA in patients with PSC may reflect the influence of toxic bile acids produced from UDCA in the colon.[6,7] Sinakos et al. determined serum bile acid composition in 56 patients with PSC previously enrolled in the Lindor et al. trial. That study has shown significant expansion of the total serum bile acid pool, including toxic LCA in the UDCA-treated group, which, in the researchers' opinion, might explain the worse outcome in these patients.[27] Therefore, we performed a detailed analysis of 29 serum bile acid species using LC-MS/MS. We observed that UDCA treatment had a significant effect not only on total concentration of serum bile acids, but also on their detailed profile. As expected, UDCA use caused an increased blood concentration of UDCA and its conjugates. In treated patients, UDCA and its species predominated with a fraction of almost half of the total serum bile acid pool, in comparison with 1.7% after drug removal. Similar phenomena have been observed in other studies, in which UDCA treatment resulted in a dose-related enrichment of both bile and serum amounts of UDCA and its species.[28-30] We also demonstrated that UDCA withdrawal exerted a

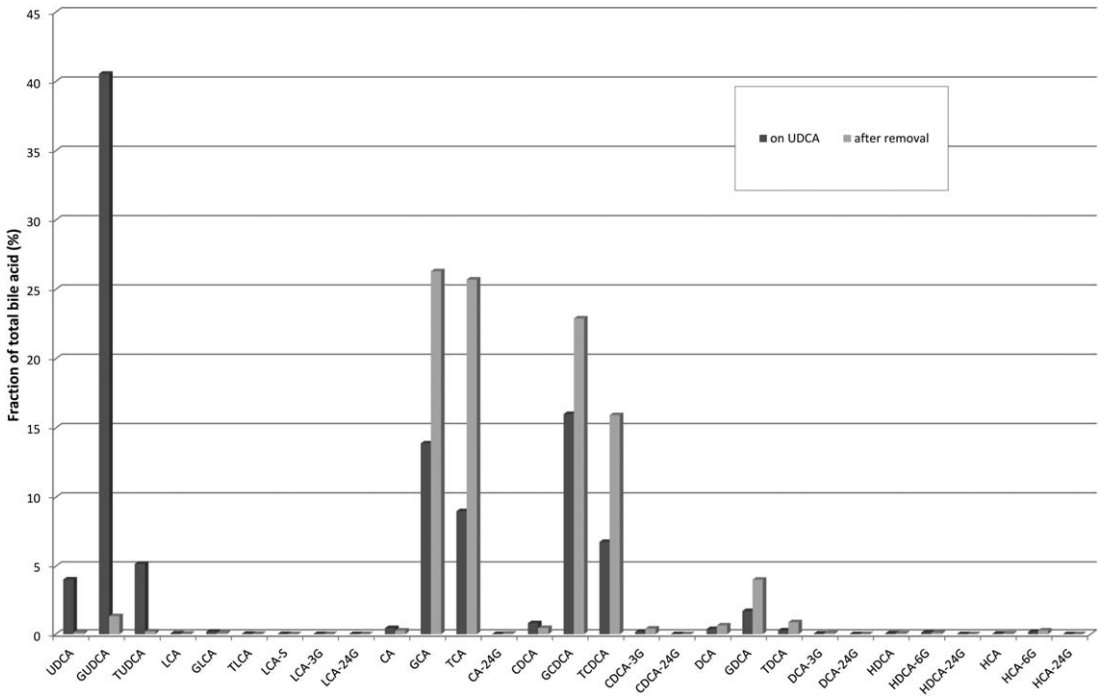


Fig. 3. Changes in mean fraction of bile acids under UDCA treatment and after drug removal. Abbreviations: UDCA, GUDCA, TUDCA, ursodeoxycholic acid and its glycine and taurine conjugates, respectively; LCA, GLCA, TLCA, LCA-3G, LCA-24G, lithocholic acid and its glycine, taurine, and glucuronide conjugates, respectively; LCA-S, sulpholithocholate; CA, GCA, TCA, CA-24G, cholic acid and its glycine, taurine, and glucuronide conjugates, respectively; CDCA, GCDCA, TCDCA, CDCA-3G, CDCA-24G, chenodeoxycholic acid and its glycine, taurine, and glucuronide conjugates, respectively; DCA, GDCA, TDCA, DCA-3G, DCA-24G, deoxycholic acid and its glycine, taurine, and glucuronide conjugates, respectively; HDCA, HDCA-6G, HDCA-24G, hyodeoxycholic acid and its glucuronide conjugates; HCA, HCA-6G, HCA-24G, hyocholic acid and its glucuronide conjugates.

significant effect on other bile acids, including LCA. UDCA implementation correlated with an extended pool of serum LCA. In fact, intestinal absorption of UDCA is incomplete and oral administration of UDCA permits bacterial 7-beta-dehydroxylation in the colon, yielding LCA.[31-33] Sinakos et al. postulated that this effect is common in high-dose treatment (i.e., 25-30 mg/kg/day).[27] The 7-alpha/beta-dehydroxylated bile acids are markedly hydrophobic and much more hepatotoxic than hydrophilic bile acids.[34] It has been suggested that LCA causes segmental bile duct obstruction, destructive cholangitis, and periductal fibrosis[35] and promotes colonic carcinogenesis.[36-38] However, the vast majority of studies relate to animal models, in which detoxification mechanisms significantly differ from those observed in humans.[39] Several studies suggest that LCA is rapidly metabolized to less toxic metabolites on the first pass through the human liver, mainly through sulfation, whereas the other species lack efficient sulfating capabilities.[40,41] Therefore, it is possible that, even by enhanced colonic production of LCA, exposure of the biliary tree to the toxic molecule may not necessary be harmful. This hypothesis is supported by our observation that serum LCA-S levels significantly dropped after UDCA removal. Thus, despite the fact that UDCA administration causes serum LCA to increase, LCA contributes a negligible amount to the bile acid pattern. On the other hand, during oral administration, UDCA becomes the major bile acid and markedly changes the balance in favor of nontoxic hydrophilic bile acids, and this change may compensate for the increased amounts in hydrophobic bile acid species.

In order to investigate whether UDCA-related changes in bile acid pattern affect the molecular mechanisms regulating bile acid synthesis, we determined serum FGF19 levels. FGF19 is the founding member of the endocrine FGF subfamily, which exerts a variety of metabolic effects influencing glucose, lipid metabolism, as well as gall bladder filling.[42-45] FGF19 plays an important role as a negative feedback regulator of hepatic bile acid synthesis, acting through the FGF receptor, 4/Klotho-$\beta$ receptor, complexes in the liver to inhibit cytochrome P7A1, a crucial enzyme in the synthesis of bile acids.[46-48] Bile acids bind to the farnesoid X receptor, stimulating FGF19 transcription. Recent work by Zhang et al. has shown that physiological concentrations of bile acids, including CDCA,

**Table 3. Correlations Between Liver Biochemistry Tests, Bile Acid Concentration, and FGF19 After UDCA Removal**

| Bile Acid Species | Correlation Coefficient (r) | *P* Value |
|---|---|---|
| ALP | 0.442 | 0.0245 |
| GGT | 0.063 | 0.7659 |
| Total bilirubin | 0.974 | <0.0001 |
| ALT | −0.019 | 0.9275 |
| AST | 0.088 | 0.6767 |
| UDCA | −0.091 | 0.6586 |
| GUDCA | 0.026 | 0.8983 |
| TUDCA | 0.095 | 0.6429 |
| LCA | −0.219 | 0.2823 |
| GLCA | −0.064 | 0.7559 |
| TLCA | 0.178 | 0.3836 |
| LCA-S | −0.082 | 0.6920 |
| CA | −0.062 | 0.7632 |
| GCA | 0.880 | <0.0001 |
| TCA | 0.954 | <0.0001 |
| CDCA | −0.144 | 0.4819 |
| GCDCA | 0.777 | <0.0001 |
| TCDCA | 0.880 | <0.0001 |
| DCA | −0.193 | 0.3447 |
| GDCA | 0.015 | 0.9212 |
| TDCA | 0.077 | 0.7072 |
| HDCA | −0.104 | 0.6114 |
| HCA | −0.115 | 0.5766 |
| Total bile acids | 0.906 | <0.0001 |

Abbreviations: UDCA, GUDCA, TUDCA, ursodeoxycholic acid and its glycine and taurine conjugates, respectively; LCA, GLCA, TLCA, lithocholic acid and its glycine and taurine conjugates, respectively; LCA-S, sulpholithocholate; CA, GCA, TCA, cholic acid and its glycine and taurine conjugates, respectively; CDCA, GCDCA, TCDCA, chenodeoxycholic acid and its glycine and taurine conjugates, respectively; DCA, GDCA, TDCA, deoxycholic acid and its glycine and taurine conjugates, respectively.

and its glycine conjugate and synthetic FXR agonist, obeticholic acid, in explants of ileal mucosa effectively stimulate FGF19 expression, whereas DCA and LCA are significantly less potent inducers.[11] In our study, we did not observe a reduction of FGF19 levels after UDCA withdrawal, despite depletion in total bile acid pool in UDCA species. Moreover, circulating FGF19 concentrations did not correlate with total and all single bile acid concentrations in UCDA treatment. However, there were significant associations between FGF19 and taurine conjugates of CA and CDCA after UDCA withdrawal, both in uni- and multivariate analysis. These results may suggest that, under cholestatic conditions, CDCA and CA may exert an inductory effect on FGF19 and that UDCA acts through FGF19-independent mechanisms.

The data on the influence of UDCA on well-being in patients with PSC are scanty. In a multicenter, randomized, controlled trial of high-dose UDCA in patients with PSC, scores for quality of life estimated by the SF-36 tool were remarkably constant during medication and did not differ between UDCA and placebo groups.[49] In our study, we did not observe

**Table 4. Quality of Life Measured by PBC-40 and SF-36 Questionnaires Before and After UDCA Withdrawal**

| | On UDCA | After Withdrawal | *P* Value |
|---|---|---|---|
| PBC-40_Other symptoms | 11.8 ± 4.5 | 11.7 ± 3.1 | >0.999 |
| PBC-40_Itch | 2.4 ± 2.1 | 3.2 ± 2.8 | 0.112 |
| PBC-40_Fatigue | 22.4 ± 9.5 | 23.6 ± 9.6 | 0.681 |
| PBC-40_Cognitive | 10.9 ± 5.2 | 10.9 ± 5.0 | 0.932 |
| PBC-40_Social and Emotional | 24.9 ± 9.3 | 26.4 ± 9.2 | 0.493 |
| SF-36_Physical Functioning | 84.4 ± 21.6 | 87.9 ± 14.5 | 0.083 |
| SF-36_Role Physical | 79.4 ± 32.3 | 70.2 ± 36.1 | 0.209 |
| SF-36_Bodily Pain | 72.0 ± 34.4 | 77.0 ± 25.1 | 0.083 |
| SF-36_General Health | 58.3 ± 24.4 | 55.0 ± 18.0 | 0.339 |
| SF-36_Vitality | 64.5 ± 17.8 | 59.5 ± 20.7 | 0.144 |
| SF-36_Social Functioning | 69.4 ± 26.3 | 82.8 ± 21.0 | 0.026 |
| SF-36_Role Emotional | 86.2 ± 27.9 | 88.5 ± 26.6 | 0.779 |
| SF-36_Mental Health | 71.8 ± 21.0 | 71.0 ± 16.9 | 0.487 |
| SF-36_Physical Component Summary | 67.3 ± 22.9 | 74.7 ± 17.9 | 0.109 |
| SF-36_Mental Component Summary | 64.6 ± 20.6 | 75.4 ± 19.1 | 0.026 |

statistically important differences in all domains of the PBC-40 questionnaire and pruritus intensity measured by NRS; however, with respect to the Mental Component Summary and Social Functioning domain of the SF-36, there was an improvement of well-being at the end of the study. In individual patients included to the study, UDCA discontinuation exerted both a negative or positive effect on HRQoL. Of note, one of the patients dropped out from the study (he returned to his UDCA medication) because of intense pruritus after UDCA removal. Interpretation of these data remains difficult and deserves more attention over the long term.

Our limitations include that we measured only serum concentration of bile acids and no data on biliary and urine analytes were available. Therefore, our study does not provide complete information about changes in bile acid equilibrium under UDCA

**Table 5. Changes in Quality of Life After UDCA Removal**

| | Improvement (%) | No Change (%) | Deterioration (%) |
|---|---|---|---|
| PBC-40_Other symptoms | 9 (34.6) | 3 (11.5) | 14 (53.9) |
| PBC-40_Itch | 4 (15.4) | 11 (42.3) | 11 (42.3) |
| PBC-40_Fatigue | 13 (50.0) | 3 (11.5) | 10 (38.5) |
| PBC-40_Cognitive | 12 (46.15) | 2 (7.7) | 12 (46.15) |
| PBC-40_Social and Emotional | 11 (42.3) | 0 | 15 (57.7) |
| SF-36_Physical Functioning | 7 (28.0) | 14 (56.0) | 4 (16.0) |
| SF-36_Role Physical | 4 (16.0) | 14 (56.0) | 7 (28.0) |
| SF-36_Bodily Pain | 10 (40.0) | 9 (36.0) | 6 (24.0) |
| SF-36_General Health | 9 (36.0) | 1 (4.0) | 15 (60.0) |
| SF-36_Vitality | 7 (28.0) | 4 (16.0) | 14 (56.0) |
| SF-36_Social Functioning | 14 (56.0) | 5 (20.0) | 6 (24.0) |
| SF-36_Role Emotional | 5 (20.0) | 17 (68.0) | 3 (12.0) |
| SF-36_Mental Health | 9 (36.0) | 4 (16.0) | 12 (48.0) |
| SF-36_Physical Component Summary | 14 (56.0) | 0 | 11 (44.0) |
| SF-36_Mental Component Summary | 16 (64.0) | 0 | 9 (36.0) |

treatment, and we are not able to interpret whenever the changes in serum LCA reflect the same changes in biliary bile acid profile. Additionally, our findings are focused on the short-term effects of UDCA withdrawal, and alongside this, the quality-of-life tools applied are not specifically designed for use in patients with PSC.

In conclusion, effective medical treatment of PSC has been hindered by uncertainty regarding the pathogenesis of the disease and the factors responsible for its progression.[7] Our findings clearly show that interruption of long-term treatment with UDCA results in rapid, significant deterioration of biochemical cholestasis. These changes are accompanied by an increased MRS. Further evaluation of patient impact on stopping UDCA in PSC is warranted, including consequences for clinical trial design.

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